VII. MECHANISMS OF TOXICITY

General Theories

The literature on the mechanism of inactivation of microorganisms by chloramines is limited. Since low levels of inorganic chloramines are effective in inactivating bacteria, Nusbaum (1952) proposed that the mechanism of action must essentially be the same as that of hypochlorous acid on enzymes; that is, the chloramine molecules enter the cytoplasm and interfere with enzymatic reations. Ingols et al. (1953) studied bactericidal mechanisms of chlorine compounds on unspecified bacterial suspensions. They noted that monochloroamine bacteriotoxicity was not completely reversed by the addition of sulfhydryl radicals; and thus, conclude that sulfhydryl radical oxidation was not the exclusive mechanism of toxicity. They proposed that the sulfhydryl group of critical enzymes may be the point of vulnerability to a strong oxidant (for example, chlorine dioxide) but that changes in other functional groups may also be involved in cell death. Thus, a weak oxidant like monochloramine may lead to microbial inactivation by changes in groups other than sulfhydryls.

Lu Shih and Lederberg (1976) showed that chloramine applied to both intact *Bacillus subtilis* cells or to the extracted bacterial DNA resulted in double and single strand breaks. Some microbial toxicity may, therefore, be attributable to DNA damage. Monochloramine produced chromosome breaks in *Vicia faba* (Fetner, 1962), and organic chloramines have been shown to produce chromosomal abnormalities in rodent cells (NIEHS, 1982).

Through observation of patients undergoing long-term hemodialysis and concomitant analysis of the water used for this process, Eaton et al. (1973) ascertained that chloramines were probably the oxidants responsible for hemolytic anemia in the patients. *In vitro* studies using human RBCs indicated that chloramine produced denaturation of hemoglobin through direct oxidant damage to erythrocytes. It also inhibited the hexose monophosphate shunt, which generates NADPH that in turn protects RBCs from oxidant damage.

A study by Grisham et al. (1984) demonstrated that organic N-chloramines formed *in vivo* play an important role in ameliorating oxidant effects but can also contribute to monochloramine formation. These workers stimulated isolated human neutrophilic leukocytes to produce hydrogen peroxide (H_2O_2) and secrete cytoplasmic granule components such as myeloperoxidase into the medium. Myeloperoxidase catalyzed the oxidation of chloride (Cl^-) by H_2O_2 to yield hypochlorous acid (HOCl). The HOCl, in turn, reacted with endogenous nitrogen compounds to yield derivatives containing nitrogen-chlorine (N-Cl) bonds, such as hydrophilic, low molecular weight, mono-N-chloramine (RNHCl) derivatives. The RNHCl derivatives were of low toxicity, but reacted with ammonium ion (NH_4^+) to yield monochloramine (NH_2Cl). The bactericidal, cytotoxic and cytolytic activities of the organic N-chloramines (RNHCl) result from reaction of RNHCl derivatives and the ammonium ion. This indicates that neutrophil amines act as a trap for HOCl, and by competing with endogenous NH_4^+ for reaction with HOCl, protect neutrophils and other cells from oxidative attack. The RNHCl derivatives remain as a

reserve of oxidizing equivalents that convert to a toxic form when an increase in NH_4^+ concentration favors formation of monochloramine.

Nusbaum (1952) proposed that the mechanism of action of dichloramine was similar to monochloramine, but there are insufficient data to support this contention. Silver et al. (1947c) demonstrated that trichloramine reacted with cystine and cysteine residues to produce a reagent that was responsible for causing canine hysteria.

Summary

Bactericidal, cytotoxic and cytolytic activities of organic N-chloramines (RNHCl) result from the reaction of RNHCl derivatives and the ammonium ion to yield monochloramine (NH_2Cl). The RNHCl derivatives remain as a reserve of oxidizing equivalents, which convert to a toxic form when the ammonium ion concentration increases favoring monochloramine formation (Grisham et al., 1984).

It has been suggested that the chloramine molecules enter the cytoplasm and interfere with enzymatic reactions (Nusbaum, 1952). Ingols et al. (1953) found monochloramine required higher concentrations and longer contact times to destroy bacteria than hypochlorous acid, suggesting that monochloramine led to microbial inactivation through enzyme changes that may not have been involved in bacterial inactivation by hypochlorous acid. Chloramines are also capable of causing DNA damage in bacterial plant and mammalian cells, which may contribute to cytotoxicity. Eaton et al. (1973) found that chloramines produced denaturation of hemoglobin through both direct oxidant damage to RBCs and inhibition of the hexose monophosphate shunt.